AMENDMENTS TO THE SPECIFICATION

Please replace the following paragraph after the section marked "Related Applications."

This application is a continuation of U.S. Patent Application Serial No. 09/576,062, filed on May 22, 2000, which issued as U.S. Patent No. 6,608,029; which is a continuation of U.S. Patent Application Serial No. 08/302,069, filed on September 7, 1994, which issued as U.S. Patent No. 6,114,304; which ~~This application~~ is a continuation-in-part of U.S. Patent Application Serial No. 08/118,381, filed September 7, 1993, now abandoned. The contents of these applications are ~~which is~~ incorporated herein by reference.

Please replace the first full paragraph beginning at page 21, line 6, with the following rewritten paragraph:

-- We have now discovered that, surprisingly in view of its previously described hyperglycemic properties, amylin and amylin agonists, including as those described herein, for example, the amylin agonist analogue ^{25,28,29}Pro-h-amylin [SEQ ID NO:1] (also referred to as "AC-0137"), can reduce gastric motility and slow gastric emptying, as evidenced by the ability of such compounds to reduce post-prandial plasma glucose levels. –

Please replace the paragraph beginning at page 24, line 5, with the following rewritten paragraph:

-- In another aspect, the invention is directed to a method of accelerating gastric emptying in a subject by administering to the subject a therapeutically effective amount of an amylin antagonist. By amylin antagonist is meant a compound which interferes with the effects of amylin, for example, a compound that is itself lacks significant pharmacological activity but causes effects by inhibition of action of a specific agonist e.g., by competition for agonist binding sites. Preferably, the amylin antagonist used in these methods is an amylin receptor antagonist. A preferred antagonist is acetyl-^{11,18}Arg, ^{30}Asn, ^{32}Tyr$^{9-32}$calcitonin (salmon) [SEQ ID NO:2]. --

Please replace the paragraph beginning at page 28, line 4, with the following rewritten paragraph:

-- The nomenclature of various amylin agonist analogue compounds useful in the present invention can be used to indicate both the peptide that the sequence is based on and the modifications made to any basic peptide amylin sequence, such as human amylin. An amino acid preceded by a superscript number indicates that the named amino acid replaces the amino acid normally present at the amino acid position of the superscript in the basic amino acid sequence. For example, "^{18}Arg^{25,28}Pro-h-amylin" [SEQ ID NO:3] refers to a peptide based on the sequence of "h-amylin" or "human-amylin" having the following substitutions: Arg replacing His at residue 18, Pro replacing Ala at residue 25 and Pro replacing Ser at residue 28. The term "des-^{1}Lys-h-amylin" [SEQ

ID NO:4] refers to a peptide based on the sequence of human amylin, with the first, or N-terminal, amino acid deleted. --

Please replace the paragraph beginning at page 29, line 3, with the following rewritten paragraph:

-- Preferred amylin agonist analogue compounds des-^{1}Lys-h-amylin [SEQ ID NO:4], ^{28}Pro-h-amylin [SEQ ID NO:5], ^{25,28,29}Pro-h-amylin [SEQ ID NO:1], ^{18}Arg^{25,28}Pro-h-amylin [SEQ ID NO:3], and des-^{1}Lys^{18}Arg^{25,28}Pro-h-amylin [SEQ ID NO:6], all show amylin activity in vivo in treated test animals, provoking marked hyper;actemia followed by hyperglycemia. In addition to having activities characteristic of amylin, certain preferred compounds have also been found to possess more desirable solubility and stability characteristics when compared to human amylin. These preferred compounds include ^{25}Pro^{26}Val^{28,29}Pro-h-amylin [SEQ ID NO:7], ^{25,28,29}Pro-h-amylin [SEQ ID NO:1] (also referred to herein as "AC-0137"), and ^{18}Arg^{25,28}Pro-h-amylin [SEQ ID NO:3]. --

Please replace the paragraph beginning at page 29, line 13, with the following rewritten paragraph:

-- The method of the present invention can employ an amylin agonist, including amylin or an amylin agonist analogue, for example, amylin receptor agonist analogues such as ^{18}Arg^{25,28}Pro-h-amylin [SEQ ID NO:3], des-^{1}Lys^{18}Arg^{25,28}Pro-h-amylin [SEQ ID NO:6], ^{18}Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:8], des-^{1}Lys^{18}Arg^{25,28,29}Pro-h-amylin [SEQ ID NO:9], ^{25,28,29}Pro-h-amylin [SEQ ID NO:1], des^{1}Lys^{25,28,29}Pro-h-amylin [SEQ ID NO:10], and ^{25}Pro^{26}Val^{28,29}Pro-h-amylin [SEQ ID NO:7]. Examples of other suitable amylin agonist analogues include:

^{23}Leu^{25}Pro^{26}Val^{28,29}Pro-h-amylin [SEQ ID NO:11];

^{23}Leu^{25}Pro^{26}Val^{28}Pro-h-amylin [SEQ ID NO:12];

des-^{1}Lys^{23}Leu^{25}Pro^{26}Val^{28}Pro-h-amylin [SEQ ID NO:13];

^{18}Arg^{23}Leu^{25}Pro^{26}Val^{28}Pro-h-amylin [SEQ ID NO:14];

^{18}Arg^{23}Leu^{25,28,29}Pro-h-amylin [SEQ ID NO:15];

^{18}Arg^{23}Leu^{25,28}Pro-h-amylin [SEQ ID NO:16];

$^{17}Ile^{23}Leu^{25,28,29}Pro$-h-amylin [SEQ ID NO:17];

$^{17}Ile^{25,28,29}Pro$-h-amylin [SEQ ID NO:18];

des-$^{1}Lys^{17}Ile^{23}Leu^{25,28,29}Pro$-h-amylin [SEQ ID NO:19];

$^{17}Ile^{18}Arg^{23}Leu$-h-amylin [SEQ ID NO:20];

$^{17}Ile^{18}Arg^{23}Leu^{26}Val^{29}Pro$-h-amylin [SEQ ID NO:21];

$^{17}Ile^{18}Arg^{23}Leu^{25}Pro^{26}Val^{28,29}Pro$-h-amylin [SEQ ID NO:22];

$^{13}Thr^{21}His^{23}Leu^{26}Ala^{28}Leu^{29}Pro^{31}Asp$-h-amylin [SEQ ID NO:23];

$^{13}Thr^{21}His^{23}Leu^{26}Ala^{29}Pro^{31}Asp$-h-amylin [SEQ ID NO:24];

des-$^{1}Lys^{13}Thr^{21}His^{23}Leu^{26}Ala^{28}Pro^{31}Asp$-h-amylin [SEQ ID NO:25];

$^{13}Thr^{18}Arg^{21}His^{23}Leu^{26}Ala^{29}Pro^{31}Asp$-h-amylin [SEQ ID NO:26];

$^{13}Thr^{18}Arg^{21}His^{23}Leu^{28,29}Pro^{31}Asp$-h-amylin [SEQ ID NO:27]; and

$^{13}Thr^{18}Arg^{21}His^{23}Leu^{25}Pro^{26}Ala^{28,29}Pro^{31}Asp$-h-amylin [SEQ ID NO:28]. --

Please replace the paragraph beginning at page 31, line 4, with the following rewritten paragraph:

-- One such method for use in identifying or evaluating the ability of a compound to slow gastric motility comprises: (a) bringing together a test sample and a test system, said test sample comprising one or more test compounds, and said test system comprising a system for evaluating gastric motility, said system being characterized in that it exhibits, for example, elevated plasma glucose in response to the introduction to said system of glucose or a meal; and, (b) determining the presence or amount of a rise in plasma glucose in said system. Positive and/or negative controls may be used as well. Optionally, a predetermined amount of amylin antagonist (e.g., $^{8-32}$salmon calcitonin [SEQ ID NO:30]) may be added to the test system. --

Please replace the paragraph beginning at page 65, line 13, with the following rewritten paragraph:

-- Uptake of an ingested labelled glucose load was measured in 11 conscious 24-hr fasted corpulent (500-600 g) LA/N rats on 2 occasions, 14 days apart. On one occasion, rats were injected subcutaneously with 3 ug of the selective amylin antagonist AC-0187 (ac-$^{10}N^{32Y8-32}$salmon calcitonin [SEQ ID NO:29], 3 minutes before gavage. On the other occasion, rats were preinjected with saline vehicle alone. Rats were gavaged with 5 uCi of [3^{3}H]glucose in 1 ml of 50% glucose. Blood samples were taken from anesthetized tails 0, 15, 30, 60, 90 and 120 minutes after gavage and were assayed for glucose-derived tritium and glucose. --

At page 28, after the first full paragraph under the heading "DETAILED DESCRIPTION OF THE INVENTION," please insert the following paragraphs:

Amylin agonist analogues useful in the methods of this application include amylin agonist analogues having the following amino acid sequence:

$^{1}A_1$-X-Asn-Thr-^{5}Ala-Thr-Y-Ala-Thr-^{10}Gln-Arg-Leu-
B_1-Asn-^{15}Phe-Leu-C_1-D_1-E_1-$^{20}F_1$-G_1-Asn-H_1-Gly-$^{25}I_1$-J_1-
Leu-K_1-L_1-^{30}Thr-M_1-Val-Gly-Ser-^{35}Asn-Thr-Tyr-Z

wherein A_1 is hydrogen Lys, Ser, Ala, des-α-amino Lys, or acetylated Lys; B_1 is Ala, Ser or Thr; C_1 is Val, Leu or Ile; D_1 is His or Arg; E_1 is Ser or Thr; F_1 is Ser, Thr, Gln or Asn; G_1 is Asn, Gln or His; H_1 is Phe, Leu or Tyr; I_1 is Ala or Pro; J_1 is Ile, Val, Ala or Leu; K_1 is Ser, Pro, Leu, Ile or Thr; L_1 is Ser, Pro or Thr; M_1 is Asn, Asp or Gln; X and Y are independently selected residues having side chains which are chemically bonded to each other to form an intramolecular linkage; and Z is hydroxy, amino, alkylamino, dialkylamino, cycloalkylamino, arylamino, aralkylamino, alkyloxy, aryloxy or aralkyloxy; provided that (a) when A_1 is Lys, B_1 is Ala, C_1 is Val, D_1 is His, E_1 is Ser, F_1 is Ser, G_1 is Asn, H_1 is Phe, I_1 is Ala, J_1 is Ile, K_1 is Ser, L_1 is Ser, and M_1 is Asn; (b) when A_1 is Lys, B_1 is Ala, C_1 is Ile, D_1 is Arg, E_1 is Ser, F_1 is Ser, G_1 is Asn, H_1 is Leu, I_1 is Ala, J_1 is Ile, K_1 is Ser, L_1 is Pro, and M_1 is Asn; (c) when A_1 is Lys, B_1 is Ala, C_1 is Val, D_1 is Arg, E_1 is Thr, F_1 is Ser, G_1 is Asn, H_1 is Leu, I_1 is Ala, J_1 is Ile, K_1 is Ser, L_1 is Pro, and M_1 is Asn; (d) when A_1 is Lys, B_1 is Ala, C_1 is Val, D_1 is Arg, E_1 is Ser, F_1 is Ser, G_1 is Asn, H_1 is Leu, I_1 is Pro, J_1 is Val, K_1 is Pro, L_1 is Pro, and M_1 is Asn; (e) when A_1 is Lys, B_1 is Ala, C_1 is Val, D_1 is His, E_1 is Ser, F_1 is Asn, G_1 is Asn, H_1 is Leu, I_1 is Pro, J_1 is Val, K_1 is Ser, L_1 is Pro and M_1 is Asn; or (f) when A_1 is Lys, B_1 is Thr, C_1 is Val, D_1 is Arg, E_1 is Ser, F_1 is Ser, G_1 is His, H_1 is Leu, I_1 is Ala, J_1 is Ala, K_1 is Leu, L_1 is Pro and M_1 is Asp; then one or more of any of A_1 to M_1 is not an L-amino acid and Z is not amino.

Suitable side chains for X and Y include groups derived from alkyl sulfhydryls which may form disulfide bonds; alkyl acids and alkyl amines which may form cyclic lactams; alkyl aldehydes or alkyl halides and alkylamines which may condense and be reduced to form an alkyl amine bridge; or side chains which may be connected to form an alkyl, alkenyl, alkynyl, ether or thioether bond. Preferred alkyl chains include lower alkyl groups having from about 1 to about 6 carbon atoms.

As used herein, the following terms have the following meanings unless expressly stated to the contrary:

The term "alkyl" refers to both straight- and branched-chain alkyl groups. The term "lower alkyl" refers to both straight- and branched-chain alkyl groups having a total of from 1 to 6 carbon atoms and includes primary, secondary, and tertiary alkyl groups. Typical lower alkyls include, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, n-pentyl, n-hexyl, and the like.

The term "aryl" refers to carbocyclic aromatic groups of 6 to 14 carbon atoms such as phenyl and naphthyl, as well as heterocyclic aromatic groups containing 1 to 3 heteroatoms (nitrogen, oxygen, sulfur, etc.) such as pyridyl, triazolopyrazine, pyrimidine and the like.

The term "aralkyl" refers to an "aryl" group of 6 to 10 carbon atoms directly attached to an "alkyl" group of 1 to 4 carbon atoms and includes for example benzyl, p-chlorobenzyl, p-methylbenzyl, and 2-phenylethyl.

The term "cycloalkyl" refers to cyclic alkyl groups of 5 to 8 carbon atoms.

Biologically active derivatives of the above agonist analogues are also included within the scope of amylin agonist analogues useful in the present invention in which the stereochemistry of individual amino acids may be inverted from (L)/S to (D)/R at one or more specific sites. Also included within the scope of amylin agonist analogues useful in the present invention are the agonist analogues modified by glycosylation of Asn, Ser and/or Thr residues.

Biologically active agonist analogues of amylin which contain less peptide character are also included in the scope of amylin agonist analogues useful in the present invention. Such peptide mimetics may include, for example, one or more of the following substitutions for -CO-NH- amide bonds: depsipeptides (-CO-O-), iminomethylenes ($-CH_2-NH-$), *trans*-alkenes (-CH=CH-), ß-enaminonitriles (-C(=CH-CN)-NH-), thioamides (-CS-NH-), thiomethylenes ($-S-CH_2-$ or $-CH_2-S-$), methylenes, and retro-amides (-NH-CO-).

The above-described amylin agonist analogues form salts with various inorganic and organic acids and bases. Such salts include salts prepared with organic and inorganic acids, for example, HCl, HBr, H_2SO_4, H_3PO_4, trifluoroacetic acid, acetic acid, formic acid, methanesulfonic acid, toluenesulfonic acid, maleic acid, fumaric acid, and camphorsulfonic acid.

Salts prepared with bases include, for example, ammonium salts, alkali metal salts (such as sodium and potassium salts), and alkali earth salts (such as calcium and magnesium salts). Acetate, hydrochloride, and trifluoroacetate salts are preferred.

The salts may be formed by conventional means, as by reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin. The above-described amylin agonist analogues include various stereoisomers. In the preferred amylin agonist analogues, the chiral centers on the peptide backbone are all S.

At page 66, after the last paragraph, please insert the following paragraphs:

To assist in understanding the present invention, the following further Examples A-S are included and describe the results of a series of experiments therein. The following examples relating to this invention should not, of course, be construed as specifically limiting the invention. Such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the present invention as hereinafter claimed.

EXAMPLE A

Preparation of ^{28}Pro-human-Amylin

Solid phase synthesis of this analogue of human ("h-") amylin using methylbenzhydrylamine anchor-bond resin and N^{α}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid hydrofluoric acid ("HF") in the presence of dimethylsulfide and anisole. The ^{28}Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid

analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)/e=3914.

EXAMPLE B

Preparation of ^{25}Pro^{26}Val^{28,29}Pro-h-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25}Pro^{26}Val^{28,29}Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: (M+1)/e=3936.

EXAMPLE C

Preparation of ^{2,7}Cyclo-[^{2}Asp,^{7}Lys]-h-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. ^{2}Asp and ^{7}Lys were introduced with Boc-^{2}Asp(Fmoc)-OH and Boc-^{7}Lys(Fmoc)-OH. Following selective side-chain deprotection with piperidine, the side-chain to side-chain (^{2}Asp-^{7}Lys) cyclization was carried out using benzotriazol-1yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent). Cyclization was as described in Di Maio, J., et al., J. Med. Chem., 33:661-667 (1990); and Felix, A.M., et al., Int. J. Pept. Prot. Res., 32:441 (1988). The ^{2,7}cyclo-[^{2}Asp,^{7}Lys]amylin-MBHA-resin obtained after cyclization was cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{2,7}cyclo-[^{2}Asp,^{7}Lys]-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. FAB mass spec: (M+1)/e=3925.

EXAMPLE D

Preparation of des-^{1}Lys-h-Amylin

Solid phase synthesis of des-^{1}Lys-h-amylin (also represented as $^{2-37}$h-amylin) using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,775.

EXAMPLE E

Preparation of ^{1}Ala-h-Amylin

Solid phase synthesis of ^{1}Ala-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{1}Ala-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,847.

EXAMPLE F

Preparation of ^{1}Ser-h-Amylin

Solid phase synthesis of ^{1}Ser-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of

Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{1}Ser-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,863.

EXAMPLE G

Preparation of ^{29}Pro-h-Amylin

Solid phase synthesis of this analogue of human amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{29}Pro-h-amylin was purified by preparative HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3916.

EXAMPLE H

Preparation of ^{25,28}Pro-h-Amylin

Solid phase synthesis of ^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,939,

EXAMPLE I

Preparation of des-^{1}Lys^{25,28}Pro-h-Amylin

Solid phase synthesis of des-^{1}Lys^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,811.

EXAMPLE J

Preparation of ^{18}Arg^{25,28}Pro-h-Amylin

Solid phase synthesis of ^{18}Arg^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{18}Arg^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,959.

EXAMPLE K

Preparation of des-^{1}Lys^{18}Arg^{25,28}Pro-h-Amylin

Solid phase synthesis of des-^{1}Lys^{18}Arg^{25,28}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained

by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys^{18}Arg^{25,28}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,832.

EXAMPLE L

Preparation of ^{18}Arg^{25,28,29}Pro-h-Amylin

Solid phase synthesis of ^{18}Arg^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{18}Arg^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^{+}$=3,971.

EXAMPLE M

Preparation of des-^{1}Lys^{18}Arg^{25,28,29}Pro-h-Amylin

Solid phase synthesis of des-^{1}Lys^{18}Arg^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys^{18}Arg^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the

structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+$=3,843.

EXAMPLE N

Preparation of ^{25,28,29}Pro-h-Amylin

Solid phase synthesis of ^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The ^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+$=3,949.

EXAMPLE O

Preparation of des-^{1}Lys^{25,28,29}Pro-h-Amylin

Solid phase synthesis of des-^{1}Lys^{25,28,29}Pro-h-amylin using methylbenzhydrylamine anchor-bond resin and N^a-Boc/benzyl-side chain protection was carried out by standard peptide synthesis methods. The ^{2,7}-[disulfide]amylin-MBHA-resin was obtained by treatment of Acm-protected cysteines with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization was achieved, the resin and side chain protecting groups were cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys^{25,28,29}Pro-h-amylin was purified by preparative reversed-phase HPLC. The peptide was found to be homogeneous by analytical HPLC and capillary electrophoresis and the structure was confirmed by amino acid analysis and sequence analysis. The product gave the desired mass ion. FAB mass spec: $(M+H)^+$=3,823.

EXAMPLE P

Preparation of des-^{1}Lys^{25}Pro^{26}Val^{28,29}Pro-h-Amylin

Solid phase synthesis of this h-amylin analogue using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods, and the ^{2,7}-[disulfide]amylin-MBHA-resin obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid. After cyclization is achieved, the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The des-^{1}Lys^{25}Pro^{26}Val^{28,29}Pro-h-amylin is then purified by preparative HPLC.

EXAMPLE Q

Preparation of [(D)-^{11}Arg]-Amylin

Solid phase synthesis of this amylin analogue using methylbenzhydrylamine anchor-bond resin and N^{a}-Boc/benzyl-side chain protection is carried out by standard peptide synthesis methods. (D)-^{11}Arg is introduced with Boc-(D)-^{11}Arg(Mtr)-OH. The ^{2,7}-[disulfide]amylin-MBHA-resin, obtained by treatment with thallium (III) trifluoroacetate in trifluoroacetic acid, is cyclized and the resin and side chain protecting groups are cleaved with liquid HF in the presence of dimethylsulfide and anisole. The [(D)-^{11}Arg]-amylin is then purified by preparative HPLC.

EXAMPLE R

Receptor Binding Assay

Evaluation of the binding of compounds to amylin receptors was carried out as follows: ^{125}I-rat amylin (Bolton-Hunter labeled at the N-terminal lysine) was purchased from Amersham Corporation (Arlington Heights, IL). Specific activities at time of use ranged from 1950 to 2000 Ci/mmol. Unlabeled peptides were obtained from BACHEM Inc. (Torrance, CA) and Peninsula Laboratories (Belmont, CA).

Male Sprague-Dawley rats (200-250) grams were sacrificed by decapitation. Brains were removed to cold phosphate-buffered saline (PBS). From the ventral surface, cuts were made rostral to the hypothalamus, bounded laterally by the olfactory tracts and extending at a 45° angle medially from these tracts. This basal forebrain tissue, containing the nucleus

accumbens and surrounding regions, was weighed and homogenized in ice-cold 20 mM HEPES buffer (20 mM HEPES acid, pH adjusted to 7.4 with NaOH at 23° C.). Membranes were washed three times in fresh buffer by centrifugation for 15 minutes at 48,000×g. The final membrane pellet was resuspended in 20 mM HEPES buffer containing 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

To measure ^{125}I-amylin binding, membranes from 4 mg original wet weight of tissue were incubated with ^{125}I-amylin at 12-16 pM in 20 mM HEPES buffer containing 0.5 mg/ml bacitracin, 0.5 mg/ml bovine serum albumin, and 0.2 mM PMSF. Solutions were incubated for 60 minutes at 23° C. Incubations were terminated by filtration through GF/B glass fiber filters (Whatman Inc., Clifton, NJ) which had been presoaked for 4 hours in 0.3% poylethyleneimine in order to reduce nonspecific binding of radiolabeled peptides. Filters were washed immediately before filtration with 5 ml cold PBS, and immediately after filtration with 15 ml cold PBS. Filters were removed and radioactivity assessed in a gamma-counter at a counting efficiency of 77%. Competition curves were generated by measuring binding in the presence of 10^{-12} to 10^{-6} M unlabeled test compound and were analyzed by nonlinear regression using a 4-parameter logistic equation (Inplot program; GraphPAD Software, San Diego).

In this assay, purified human amylin binds to its receptor at a measured IC_{50} of about 50 pM. Results for test compounds are set forth in Table I, showing that each of the compounds has significant receptor binding activity.

EXAMPLE S

Soleus Muscle Assay

Evaluation of the amylin agonist activity of compounds was carried out using the soleus muscle assay as follows. Male Harlan Sprague-Dawley rats of approximately 200g mass were used in order to maintain mass of the split soleus muscle less than 40mg. The animals were fasted for 4 hours prior to sacrifice by decapitation. The skin was stripped from the lower limb which was then pinned out on corkboard. The *tendo achilles* was cut just above *os calcis* and *m. gastrocnemius* reflected out from the posterior aspect of the tibia. *M. soleus*, a small 15-20mm long, 0.5mm thick flat muscle on the bone surface of *m. gastrocnemius* was then stripped clear

and the perimysium cleaned off using fine scissors and forceps. *M. soleus* was then split into equal parts using a blade passed antero-posteriorly through the belly of the muscle to obtain a total of 4 muscle strips from each animal. After dissecting the muscle from the animal, it was kept for a short period in physiological saline. It was not necessary that the muscle be held under tension as this had no demonstrable effects on radioglucose incorporation into glycogen.

Muscles were added to 50mL Erlenmeyer flasks containing 10mL of a pregassed Krebs-Ringer bicarbonate buffer containing (each liter) NaCl 118.5 mmol (6.93g), KCl 5.94 mmol (443mg), $CaCl_2$ 2.54 mmol (282mg), $MgSO_4$ 1.19 mmol (143mg), KH_2PO_4 1.19 mmol (162mg), $NaHCO_3$ 25 mmol (2.1g), 5.5 mmol glucose (1g) and recombinant human insulin (Humulin-R, Eli Lilly, IN) and the test compound, as detailed below. pH at 37° C was verified as being between 7.1 and 7.4. Muscles were assigned to different flasks so that the 4 muscle pieces from each animal were evenly distributed among the different assay conditions. The incubation media were gassed by gently blowing carbogen (95% O_2, 5% CO_2) over the surface while being continuously agitated at 37° C in an oscillating water bath. After a half-hour "preincubation" period, 0.5 μCi of U-^{14}C-glucose was added to each flask which was incubated for a further 60 minutes. Each muscle piece was then rapidly removed, blotted and frozen in liquid N_2, weighed and stored for subsequent determination of ^{14}C-glycogen.

^{14}C-glycogen determination was performed in a 7 mL scintillation vial. Each frozen muscle specimen was placed in a vial and digested in 1 mL 60% potassium hydroxide at 70° C for 45 minutes under continuous agitation. Dissolved glycogen was precipitated out onto the vial by the addition of 3 mL absolute ethanol and overnight cooling at -20° C. The supernatant was gently aspirated, the glycogen washed again with ethanol, aspirated and the precipitate dried under vacuum. All ethanol is evaporated to avoid quenching during scintillation counting. The remaining glycogen was redissolved in 1 mL water and 4 mL scintillation fluid and counted for ^{14}C.

The rate of glucose incorporation into glycogen (expressed in μmol/g/hr) was obtained from the specific activity of ^{14}C-glucose in the 5.5 mM glucose of the incubation medium, and the total ^{14}C counts remaining in the glycogen extracted from each muscle. Dose/response curves were fitted to a 4-parameter logistic model using a least-squares iterative routine (ALLFIT, v2.7, NIH, MD) to derive EC_{50}'s. Since EC_{50} is log-normally distributed, it is

expressed ± standard error of the logarithm. Pairwise comparisons were performed using *t*-test based routines of SYSTAT (Wilkinson, "SYSTAT: the system for statistics," SYSTAT Inc., Evanston IL (1989)).

Dose response curves were generated with muscles added to media containing 7.1 nM (1000μU/mL) insulin and each test compound added at final (nominal) concentrations of 0, 1, 3, 10, 30, 100, 300 and 1000 nM. Each assay also contained internal positive controls consisting of a single batch of archived rat amylin, lyophilized and stored at -70° C.

Human amylin is a known hyperglycemic peptide, and EC_{50} measurements of amylin preparations in the soleus muscle assay range typically from about 1-10 nM, although some commercial preparations which are less than 90% pure have higher EC_{50}'s due to the presence of contaminants that result in a lower measured activity. Results for test compounds are set forth in Table I, showing that each of the compounds has amylin activity.

TABLE I

		Receptor Binding Assay IC_{50} (pM)	Soleus Muscle Assay EC_{50} (nM)
1)	^{28}Pro-h-Amylin	15.0	2.64
2)	^{25}Pro^{26}Val^{28,29}Pro-h-Amylin	18.0	4.68
3)	^{2,7}Cyclo-[^{2}Asp,^{7}Lys]-h-Amylin	310.0	6.62
4)	$^{2-37}$h-Amylin	236.0	1.63
5)	^{1}Ala-h-Amylin	148.0	12.78
6)	^{1}Ser-h-Amylin	33.0	8.70
7)	^{29}Pro-h-Amylin	64.0	3.75
8)	^{25,28}Pro-h-Amylin	26.0	13.20
9)	des-^{1}Lys^{25,28}Pro-h-Amylin	85.0	7.70
10)	^{18}Arg^{25,28}Pro-h-Amylin	32.0	2.83
11)	des-^{1}Lys^{18}Arg^{25,28}Pro-h-Amylin	82.0	3.77
12)	^{18}Arg^{25,28,29}Pro-h-Amylin	21.0	1.25
13)	des-^{1}Lys^{18}Arg^{25,28,29}Pro-h-Amylin	21.0	1.86
14)	^{25,28,29}Pro-h-Amylin	10.0	3.71
15)	des-^{1}Lys^{25,28,29}Pro-h-Amylin	14.0	4.15